

iGEM TU/e 2014

Biomedical Engineering

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Creating circular RCA template

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1 Creating circular DNA

Make sure that single strand DNA template being used is 3'-hydroxylated and 5'-phosphorylated.

- Combine the following reaction components in a PCR tube:

Component	Quantity/mass/final concentration	Volume (μL)
H ₂ O	To 200 μL	x μL
10x CircLigase II buffer	1x	2 μL
ssDNA	0.5 μM	y μL
50 mM MnCl ₂	2.5 mM	1 μL
CircLigase II (200 U)	10 U/μL	2 μL
<i>Total</i>		20 μL

- Run the following program in a thermo cycler

Step	Temperature (°C)	Time (min)
Ligase activity	60	60
Heat inactivation	80	10
Cooling	4	Hold

2 Removal of linear DNA from mixture

- Add 1000 U (10 μL) of Exonuclease III to the reaction mixture
- Add 100 U (5 μL) of Exonuclease I to the reaction mixture
- Program the thermo cycler as below

Step	Temperature (°C)	Time (min)
Digestion	37	60
Heat inactivation	80	20
Cooling	4	Hold